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(54) Defining a pharmacophore for the design of MDR modulators

(57) Determining a pharmacophore for identifying (e.g. by screening) and designing molecules which possess multi-drug resistance (MDR) modulatory activity. The pharmacophore represents a 3-dimensional aggregate array of positions in space of the field points of a series of molecules having the same, or similar, MDR modulatory activity, and describes regions of positive or negative maxima in the local electrostatic field associated with a molecule, and van der Waals surfaces. A number of lowest energy conformations for each molecule are determined and selected, and interconformational comparisons between conformers of pairs of molecules are carried out and ranked in order of Coulombic overlay energy. A conformational mapping joins pairs of conformers in the top three ranked comparisons (fig. 3), and an optimum cyclic path is determined. This joins all the molecules, with their conformations selected such that the total Coulombic overlay energy over the path is minimised (fig. 4). The field points of these "active" conformations on the cyclic point are combined using an aggregate average method, to generate the pharmacophore.

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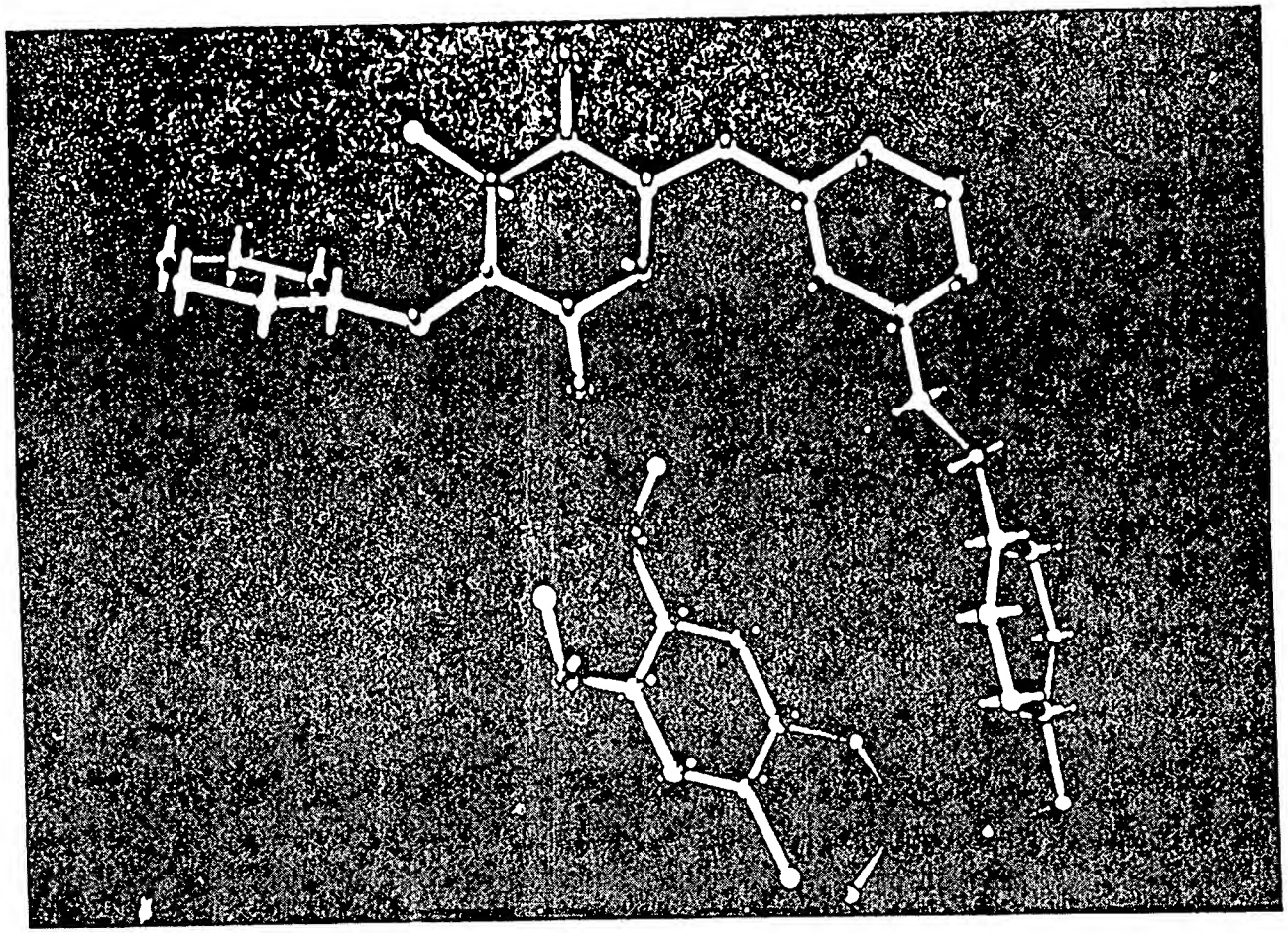


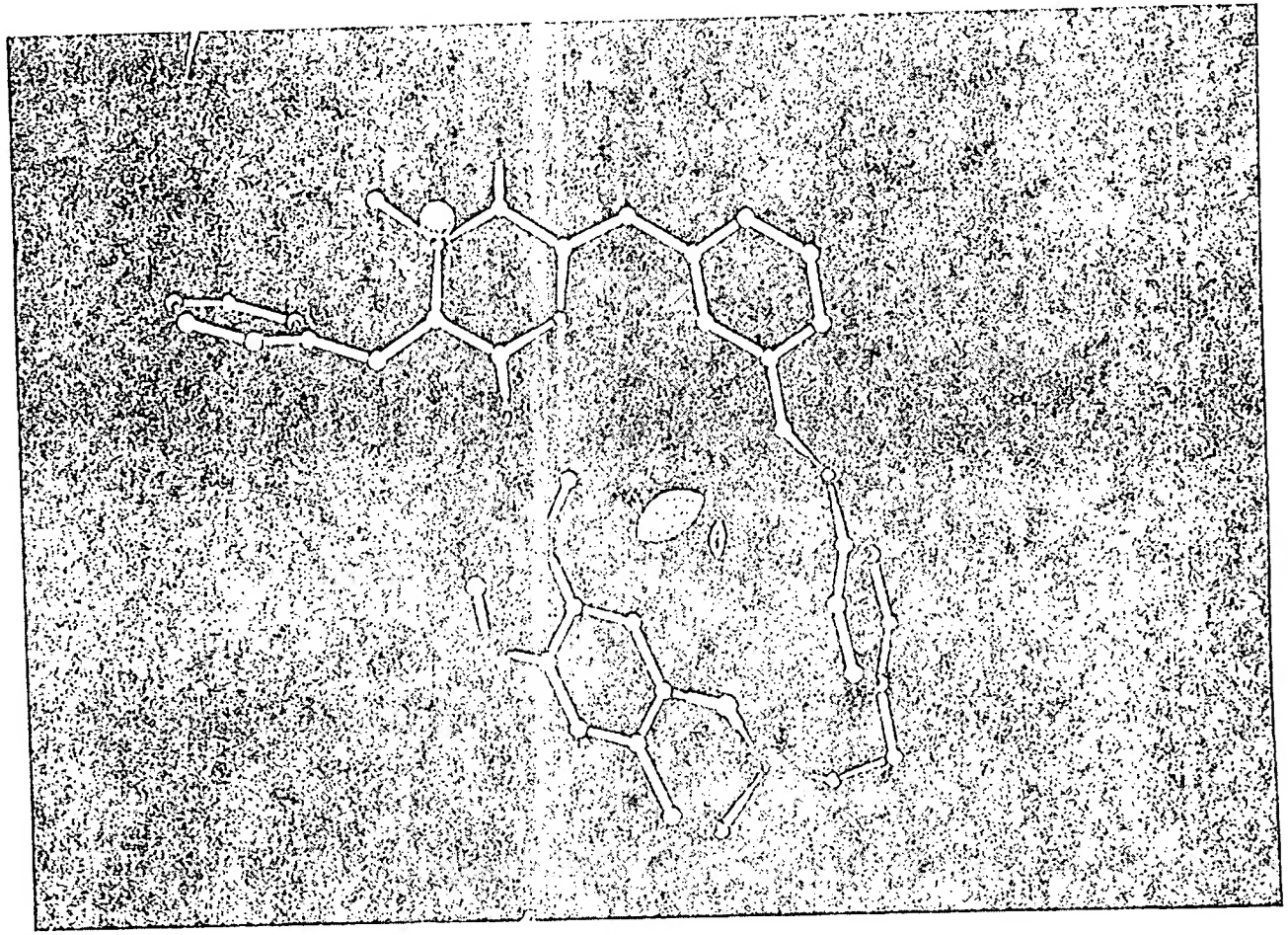
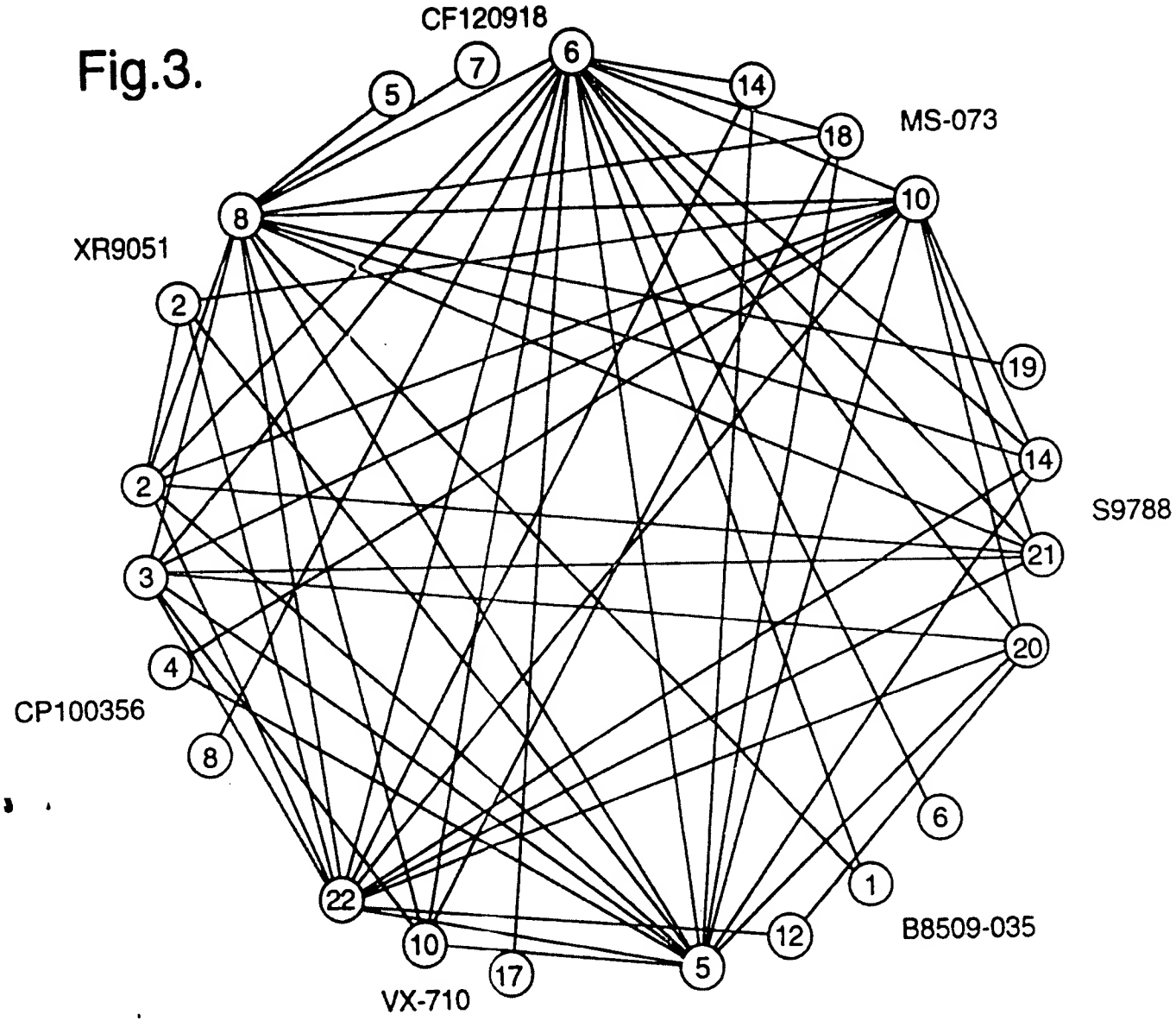


Fig.3.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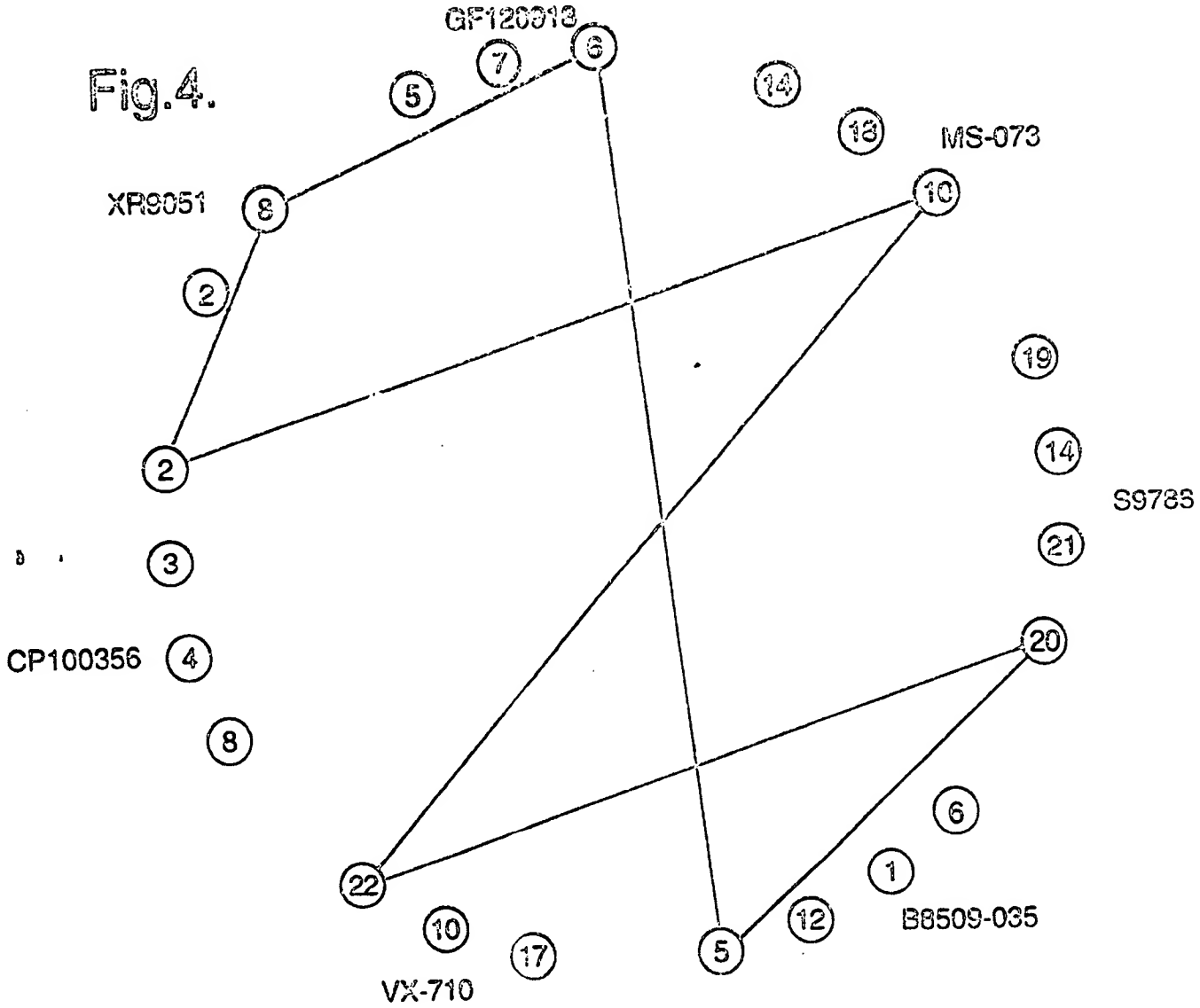
6
7
5
14
18
MS-073
10
8
XR9051
2
19
14
2
S9788
21
3
20
4
CP100356
8
6
1
22
12
B8509-035
10
17
5
VX-710

Fig.4.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7
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5
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MS-073
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2
19
2
14
S9783
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3
20
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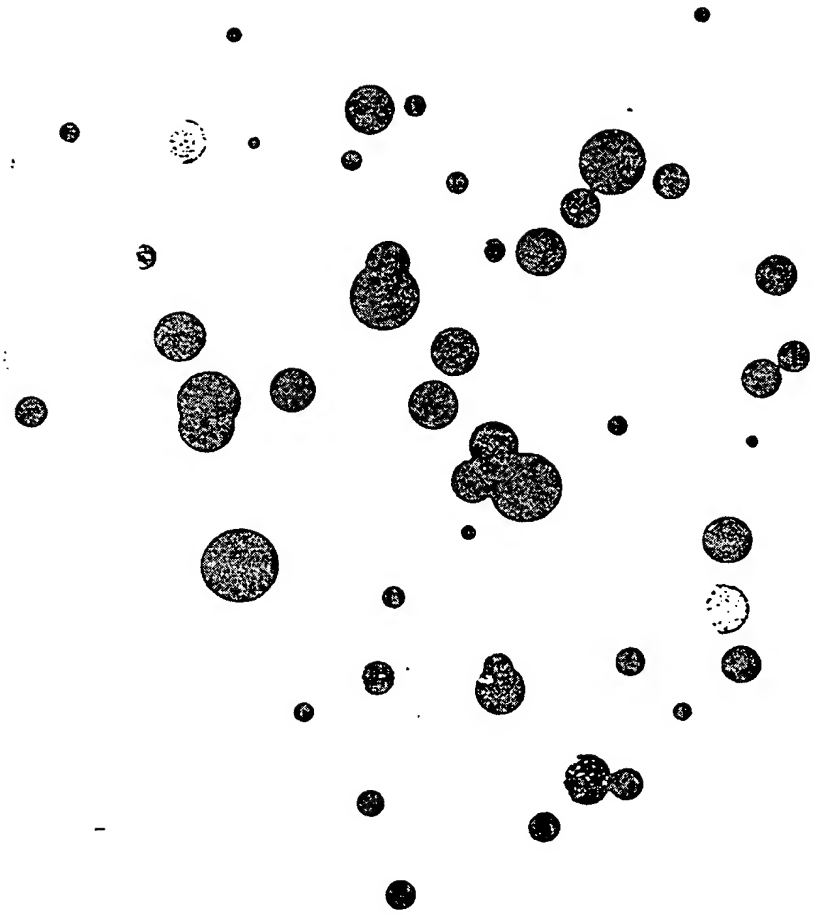
4
8
6
1
22
12
B8509-035
10
17
5
VX-710

FIGURE 5

5/6



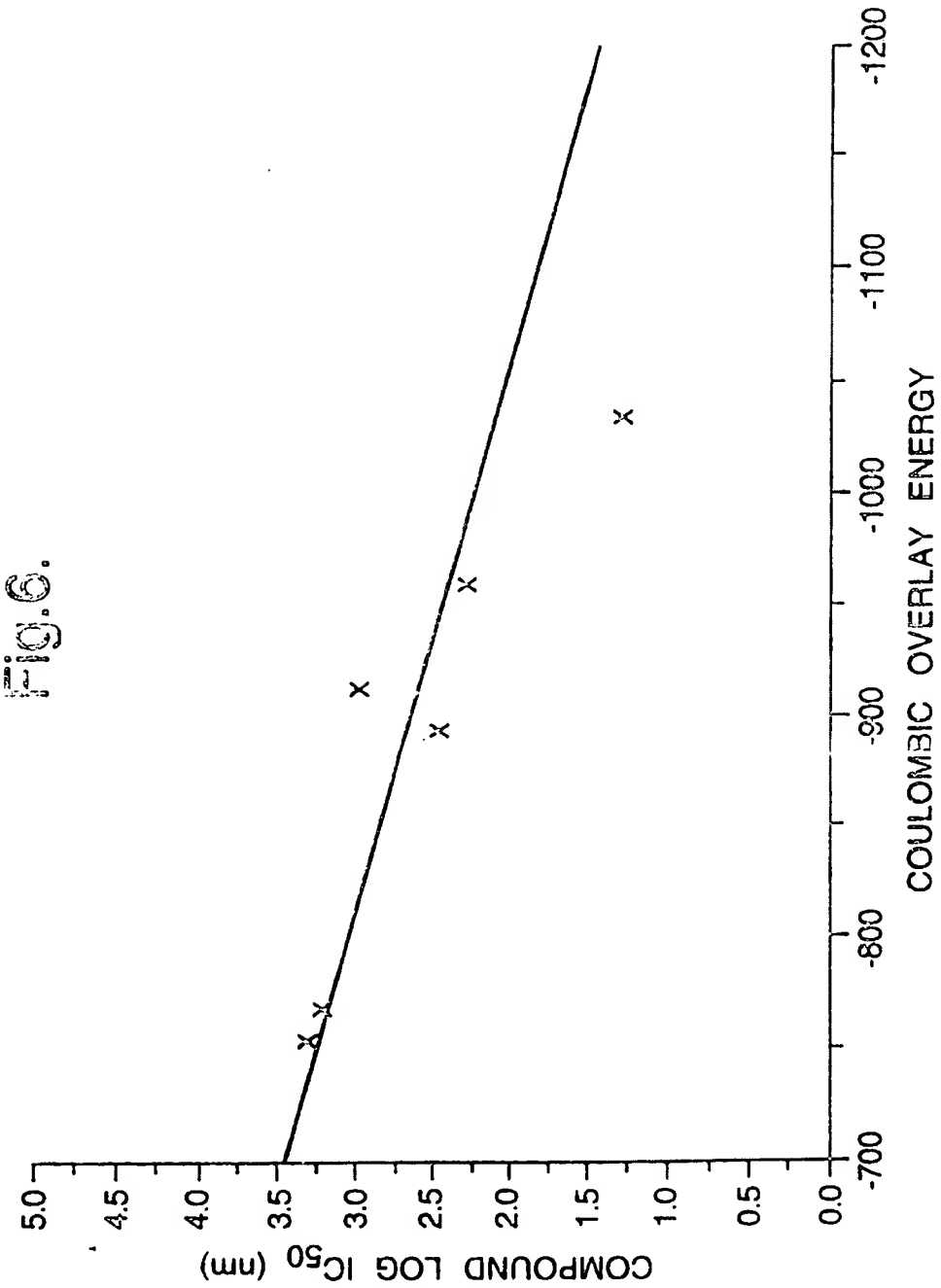

COMPOUND LOG IC_{50} (nm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-700
-800
-900
-1000
-1100
-1200
COULOMBIC OVERLAY ENERGY

Fig. 6.

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DESIGN OF MDR MODULATORS

The present invention relates to pharmacophores which are useful for identifying and designing molecules that possess multi-drug resistance (MDR) modulatory activity.

The clinical resistance of tumours to treatment with certain cytotoxic agents is an obstacle to the successful chemotherapeutic treatment of cancer patients. A tumour may acquire resistance to a cytotoxic agent used in a previous treatment. A tumour may also manifest intrinsic resistance, or cross-resistance, to a cytotoxic agent to which it has not previously been exposed, that agent being unrelated by structure or mechanism of action to any agent used in previous treatments of the tumour.

Analogously, certain pathogens may acquire resistance to pharmaceutical agents used in previous treatments of the diseases or disorders to which those pathogens give rise. Pathogens may also manifest intrinsic resistance, or cross resistance, to pharmaceutical agents to which they have not previously been exposed. Examples of this effect include multi-drug resistant forms of malaria, tuberculosis, leishmaniasis and amoebic dysentery.

The above phenomena are referred to collectively as multi-drug resistance (MDR). A 170KDa plasma membrane glycoprotein (P-gp) is implicated in the mechanism which underlies MDR.

Cancer cells which exhibit multi-drug resistance, referred to as MDR cells, display a reduction in intracellular drug accumulation compared with the corresponding drug-sensitive cells. Studies using in vitro derived MDR cell lines have shown that MDR is often associated with increased expression of P-gp which has drug binding properties. P-gp is thought to function as an efflux pump for many hydrophobic compounds, and transfection studies using cloned P-gp have shown that its overexpression can confer the MDR phenotype on cells; see, for example, Ann. Rev. Biochem 58 137-171 (1989).

A major function of P-gp in normal tissues is to export intracellular toxins from the cell. There is evidence

to suggest that overexpression of P-gp may play a clinical role in multi-drug resistance. Increased levels of P-gp mRNA or protein have been detected in many forms of human cancers - leukaemias, lymphomas, sarcomas and carcinomas. Indeed, in some cases P-gp levels have been found to be increased in tumour biopsies obtained after relapse from chemotherapy.

Inhibition of P-gp function in P-gp mediated MDR has been shown to lead to a net accumulation of anti-cancer agent in the cells. For example, Verapamil a known calcium channel blocker was shown to sensitise MDR cells to Vinca alkaloids in vitro and in vivo: Cancer Res., 41, 1967-1972 (1981). The proposed mechanism of action of the MDR modulatory compound involves competition with the anti-cancer agent for binding to the P-gp.

The MDR modulatory compounds can therefore be used in a method of potentiating the cytotoxicity of an agent which is cytotoxic to a tumour cell. Such a method comprises, for instance, administering an MDR modulatory compound to the tumour cell whilst the tumour cell is exposed to the cytotoxic agent in question. The therapeutic effect of a chemotherapeutic, or antineoplastic, agent may thus be enhanced. The multi-drug resistance of a tumour cell to a cytotoxic agent during chemotherapy may be reduced or eliminated.

Modulatory compounds can also be used in a method of treating a disease in which the pathogen concerned exhibits multi-drug resistance, for instance multi-drug resistant forms of malaria (Plasmodium falciparum), tuberculosis, leishmaniasis and amoebic dysentery. Such a method comprises, for instance, administering an MDR modulatory compound with (separately, simultaneously or sequentially) the drug to which the pathogen concerned exhibits multi-drug resistance. The therapeutic effect of the drug may thus be enhanced.

A human or animal patient harbouring a tumour may be treated for resistance to a chemotherapeutic agent by a method comprising the administration thereto of an MDR modulatory compound. The MDR modulatory compound is administered in an amount effective to potentiate the

cytotoxicity of the said chemotherapeutic agent. Examples of chemotherapeutic or antineoplastic agents which are preferred in the context of the present invention include Vinca alkaloids such as vincristine and vinblastine; anthracycline antibiotics such as daunorubicin and doxorubicin; mitoxantrone; actinomycin D; taxanes e.g. taxol; epipodophyllotoxins e.g. etoposide and plicamycin.

In addition, a human or animal patient suffering from a disease in which the responsible pathogen exhibits multi-drug resistance may be treated for resistance to a therapeutic agent by a method comprising the administration thereto of an MDR modulatory compound.

Examples of such disease include multi-drug resistant forms of malaria (Plasmodium falciparum), tuberculosis, leishmaniasis and amoebic dysentery.

MDR modulators also have utility in the delivery of drugs across the blood-brain barrier, and in the treatment of AIDS and AIDS-related complex. A human or animal patient in need of such treatment may be treated by a method comprising the administration thereto of an MDR modulatory compound.

Disadvantages of drugs which have been used to modulate MDR, termed resistance modifying agents or RMAs, include that they frequently possess a poor pharmacokinetic profile and/or are toxic at the concentrations required for MDR modulation.

Molecules that possess MDR modulatory activity span a diverse range of structural types.

The elucidation of which functional group or collection of functional groups bestows a certain biological activity on a small organic molecule has long been of interest to the pharmaceutical industry. Historically, however, the lack of satisfactory answering has relegated the development of active compounds to good fortune or exhaustive synthesis and biological testing of a large number of compounds. With the advent of computer aided drug design this situation is beginning to change. As an increasing amount of information is being gained about drug receptors and the conformations that are energetically accessible to a drug molecule at normal (physiological) temperature, a more

complete understanding is being developed of the drug - receptor interaction.

It is well understood that, on binding, molecules recognise each other by the molecular potential fields that they generate as they approach one-another. The field is not only dependent on the molecular structure but also on the molecule's conformation on approach to the receptor molecule and the induced field changes that the approach causes. Thus, it has been suggested that field correspondence rather than structural correspondence should be a guide to similarity between active molecules.

Attempts to define molecular fields in terms of electrostatic parameters have been made, for example, using CoMFA and QSAR techniques. These are restricted in the number of conformations they can handle, are bound to fixed grids and ignore interstices or use energy calculations parameterised for isolated molecules. They are compatutionally demanding and can not generally account for a diverse range of structures having the same bioactivity. Thus, using such methods, a reliable pharmacophore has not yet been developed, particularly not for MDR activity.

The invention provides a pharmacophore comprising a 3-dimensional array of field points defining a shape and volume wherein the set of 3-dimensional field points is the aggregate average of the field points derived from a plurality of molecules which possess MDR modulatory activity.

The invention also provides a method of screening a molecule for MDR modulatory activity, which method comprises;

(i) obtaining field points describing the positive, negative and van der Waals surface around the molecule;

(ii) measuring the energy of overlay when the molecular field points obtained in (i) are fitted into a pharmacophore of the invention; and

(iii) selecting the molecule as a candidate MDR modulator if the energy of overlay measured in (ii) is equal to or lower than the energy of overlay between field points for molecules known to possess MDR modulatory activity and the said pharmacophore.

The invention also provides a method of designing a

molecule having MDR modulatory activity, which method comprising the step of determining the structure of a compound which has field points that have, when fitted into a pharmacophore of the invention, an energy of overlay equal to or lower than a predetermined value.

The invention also provides a compound which possesses MDR modulatory activity identified using a pharmacophore of the invention or by a method of the invention, or designed using a method of the invention.

A pharmacophore can be thought of in general terms as any 3 dimensional array of descriptors which go towards explaining the therapeutic activity of a drug or receptor.

A pharmacophore as used herein, is defined as a three dimensional array of field points defining a shape and volume. The field points are indicative of areas of positive charge, areas of negative charge and areas of van der Waals interactions that are present in the field points derived from active compounds.

By positive field point is meant a point or area in space around a molecule which has electrophilic properties. A positive field point is most likely to interact with a negative field point on another molecule, for instance on binding to a receptor protein.

By negative field point is meant a point or area in space around a molecule which has nucleophilic properties. A negative field point is most likely to interact with a positive field point on another molecule, for instance on binding to a receptor protein.

By van der Waals surface field point is meant a lipophilic region that has a strong possibility of close approach to the receptor macromolecule in this case P-gp. By close approach it is meant a distance such that van der Waals forces are able to come into effect.

Fig 1. Depicts a conformation of XR9051 with XED charges added.

Fig 2. Depicts a conformation of XR9051 with related field points. Field colours being:
Red = positive,
green = negative and

yellow = van der Waals regions.

Fig 3. Shows a conformational mapping diagram describing the top 3 links between molecules.

Fig 4. Depicts the optimum cyclic path and related conformers.

Fig 5. Depicts a representation of a typical pharmacophore of the invention.

Fig 6. Shows a plot of log [accumulation IC_{50}] against coulombic overlay energy for a number of known MDR modulatory compounds.

Pharmacophores have previously been defined as three dimensional arrangements of chemical groups related to a given biological activity which enables meaningful comparison of molecules exhibiting the same biological function (Naruto et al. *Eur. J. Med Chem.* 20:529.532 (1985)). Pharmacophores thus defined can be derived by simple overlap of active structures or common functional groups in the molecules. However, a problem is encountered when molecules which exhibit a great diversity of structures and structural motifs are proved to be active for the same biological function. Simply overlapping the active structures or common functional groups in the molecules does not produce a reliable pharmacophore. Simple pharmacophores such as these frequently do not take into account the conformation of, or energetic accessibility to, the 'active' molecule. Without a way to orient the molecules e.g., based upon fit with another macromolecule - a receptor, enzyme, or in this case P-gp it is difficult, and in some cases impossible, to construct a reliable pharmacophore. This problem results, at least in part, from the fact that sometimes even closely related active molecules fit into macromolecules in very different ways.

In the present invention a pharmacophore is a three dimensional array of field points. Therefore a pharmacophore may be thought of as representing an aggregate array of positions in space of the field points of a series of molecules having the same or similar biological activity. Pharmacophores can be specific for different compounds, their related molecules and a particular biological activity. In

the present invention the pharmacophore is specific for MDR activity. It should be emphasized that these created pharmacophores do not exist as such in nature and are the product of aligning the field points of a plurality of molecules.

A pharmacophore, once created, stands alone and is subsequently independent from the molecules, in the present case MDR modulatory molecules, that were involved in its formation. Thus, after formation of the pharmacophore, one no longer needs to use structural comparison with the molecules themselves for the design or identification of biologically active molecules. A pharmacophore of the invention can be used to identify and design new molecules that will possess the same or similar structural and charge features that are represented by the pharmacophore. This is a completely different concept from the one of using the simple structural similarities between active molecules as the basis for the design of compounds. The pharmacophore can be used itself for any number of applications, including but not limited to the following: as a screening tool for drug development and to determine if a particular compound will possess bioactivity of a certain type, in the present case MDR modulatory activity.

Each pharmacophore has characteristic constraints placed upon its shape, topology, volume, and electrostatic profile. A pharmacophore is accurately described by its three dimensional array of field points which is represented by a coordinate system that is configured in a computer memory. Each specific atom within a molecule that fits in a pharmacophore has a specific location relative to the active site of the macromolecule with which it is to interact. The individual atoms also have electrical fields assigned to them. These fields are represented numerically and through many other ways including the use of colours and shading to indicate the field strength. As the degree of steric and electrostatic fit between the pharmacophore and the field points derived from a candidate molecule, whether said candidate molecule has previously been synthesised or not, increases, a more negative coulombic overlay energy results.

This indicates an increase in the efficacy of the pharmacophore for that candidate molecule. This could manifest itself as increased bioactivity.

The term "coulombic overlay energy" as used herein is representative of the total energy of fitting between two field patterns, each field pattern being a field point descriptor file. Thus the greater the negative value of coulombic overlay energy, the better the fit of the two field patterns. A successful pharmacophore would be expected to produce a good correlation between the coulombic overlay energy when a molecule is fitted into the pharmacophore and the MDR modulatory activity of the molecule.

The pharmacophores of the present invention can be generated using any commercial or research software capable of the processes defined below. Pharmacophores of the invention are preferably generated using the "cosmic" molecular modelling suite comprising "xedminlin", "xedfldprep", "xedmin01", "xedqmfcom", "proc_bmf", "xedsort" and "xeddraw" running on a computer suitable for running molecular modelling software being equipped with appropriate accessories. Preferably Silicon Graphics Indy, R4400, 150MHZ, 64MB RAM, 2GB HD equipped with XZ Graphics running IRIX 5.3 operating system.

The "cosmic" modelling suite is copyrighted to Dr J G Vinter, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, England, CB2 1EW.

Pharmacophores of the present invention are derived from the structures of molecules which are known to possess MDR modulatory activity preferably molecules which are known to possess potent MDR modulatory activity, more preferably molecules known to inhibit multi-drug resistance *in vivo* and/or *in vitro* and are more preferably still molecules including at least one of XR9051, MS-073, GF120918, CP100356, S9788, VX-710 and B8509-035.

The 3-dimensional array of field points of which the pharmacophores of the invention are comprised are derived from the structures of the pluarity of molecules which possess MDR modulatory activity preferably by:

(i) subjecting each molecule to conformational hunting

to generate a plurality of conformers for each molecule;

(ii) applying extended electron density (XED) charges to each molecular conformer generated in (i);

(iii) defining field points describing the positive, negative and van der Waals surface around each conformer;

(iv) making an intermolecular energy comparison between the field points defined in (iii);

(v) obtaining the three lowest energy comparisons for each intermolecular comparison and mapping them on a conformational mapping diagram;

(vi) defining an optimum cyclic path which relates the molecules; and

(vii) defining the field points for the conformer of each molecule which lies on the optimum cyclic path defined in (vi).

The structures of the plurality of molecules known to possess MDR modulatory activity are provided via construction with a chemical structure drawing program preferably compatible directly or indirectly with the torsional minimization and field preparation program "xedminlin". Preferably the structures are provided via the program "xeddraw" as cosmic.dat files. Each molecule is independently subjected to conformational hunting preferably using the program "xedminlin".

If the program "xedminlin" is used then:

the bond torsion increment angle is preferably 5-360°; more preferably 10°;

carbon tc carbon double bonds are preferably set as non rotatable bonds;

amide carbon to nitrogen bonds are preferably set as trans and as non rotatable;

if a flexible ring structure is present then the number of randomisations of that ring is preferably 5;

the upper limit of energy above the global minimum is preferably 20 Kcal;

the number of randomisations of the whole molecule is preferably 200; and

the dielectric constant is preferably 1 to 4, more preferably 1.

The results of this process are a set of energy minimized conformations for each molecule. If using "xedminlin" then these are presented in a cosmic.bst file.

Extended electron density (XED) charges are then added to each of the conformers of each of the molecules preferably using the program "xedfldprep" which is called automatically by "xedminlin". Further information about the calculation of XED charges from first principles can be found in J.G. Vinter, J.Comput.-Aided Mol.Design 8 (1994) 653-668 and J.G. Vinter et al, J.Comput.-Aided Mol.Design 9 (1995) 297-307 and references cited therein.

Field points are then defined for each of the conformations of each of the molecules preferably using the program "xedfldprep".

If "xedfldprep" is used to generate field points for each conformation then the probe used in field generation is preferably set to "peptide/peptide interface".

The information defining each field point comprises the type of field point; positive field point, negative field point and van der Waals surface field point; the 3D co-ordinates defining the position of the field point and a numerical value quantifying the size/extent of the field point.

A number of lowest energy conformations for each molecule are selected for intermolecular comparisons. The number of conformations is preferably selected in the following way:

(i) Selecting the 40 lowest energy conformations for each molecule, then

(ii) Selecting only the compounds from (i) which have an energy of 8 Kcal above the global minimum (lowest energy conformer) for the molecule then

(iii) reducing the upper energy limit to 3 Kcal above the global minimum, and then,

(iv) further raising or reducing the upper energy limit so that a convenient number of conformers are selected. The number should be chosen so that the intermolecular comparison that follows can be carried out in a practical amount of time.

Most preferably the number of conformations selected for each molecule is from 5 to 25 e.g. from 7 to 21.

If using "xedfldprep" then the conformation with XED changes and field points added is generated as a cosmic.fst file.

Comparisons between pairs of molecules are then made solely on the basis of the field point descriptors (type, location, extent) for each conformation of each of the molecules. The comparisons are preferably made using the method of least squares to establish the best fit between the field points of each of the two conformers. The comparisons are preferably made using the program "xedqmfcom" which is called automatically on selecting the BCOM menu item from the "xedfldprep" program. When using "xedqmfcom" comparisons are quantified by coulombic overlay energy. This represents the fit of one set of field points over the other. The better the fit the lower the coulombic overlay energy. For each intermolecular comparison the 50 having the lowest coulombic overlay energy are selected.

If using "xedqmfcom" then the data is generated as a cosmic.bmf file.

The results of the comparisons are then processed, preferably using the program "proc_bmf". If using "proc_bmf" the result of each intermolecular comparison between each conformer is displayed as coulombic overlay energy.

If using "proc_bmf" to process the comparison data then a text file displaying the comparisons is generated; a cosmic.com. Also an equivalent file is created automatically which is compatible with the next stage of pharmacophore design; a cosmic.sot file.

Interconformational comparisons between conformers of pairs of molecules are ranked in order of coulombic overlay energy, lowest first. The results of the comparisons are mapped onto a conformational mapping diagram. A more simple conformational mapping diagram can be obtained if only the top three ranking comparisons are mapped. Each conformer selected for use in the intermolecular comparisons is assigned a number. Numbers representing the conformers which are in the top three ranked comparisons for each pair of

compounds are placed in a pattern or shape e.g. around a circle. Pairs of conformers which are in the top three ranked comparisons are joined, each line representing a coulombic overlay energy for the comparisons of that pair of conformers. (see figure 3). The conformational mapping is carried out using a suitable mapping program, preferably the program "xedsort". When using the program "xedsort" it is preferable to select the top 3 ranked comparisons between each molecule.

The optimum cyclic path between molecules which relates specific conformations is then defined using a suitable computer program, preferably "xedsort".

A cyclic path is a path which can be followed on the conformational mapping diagram between all of the molecules represented going from one to another wherein one conformation of each molecule lies on the path. The optimum cyclic path is the cyclic path which has the lowest sum of coulombic overlay energies. The optimum cyclic path is found automatically if "xedsort" is used. In this case all cyclic paths are found and their total coulombic overlay energies calculated. The lowest is then selected (see figure 4).

The conformations that fall on this optimum cyclic path are termed the "active" conformations of each of the molecules.

The field points for each conformation which lies on the optimum cyclic path are thus known. If using the cosmic modelling suite then each conformation is located in a cosmic.fsc file.

A pharmacophore is then generated by combining the field points of the "active" conformations using an aggregate average method. The parameters, such as the area over which a field point is averaged, can be adjusted to define differing pharmacophores. The pharmacophore is generated using a suitable computer program, preferably the "create template" feature of the "xedsort" program. When using the "xedsort" program for generating the pharmacophore the preferred parameters are:

Electronic Hunting Distance (EHD) = 2.0 to 3.0

Surface Hunting Distance (SHD) = 0.5 to 3.0

Lower Limit Filter (LLF) = 2.0 to 5.0
most preferably EHD = 3.0, SHD = 3.0 and
LLF = 2.0

The pharmacophore is generated as a cosmic.cpf file. The most preferred pharmacophore is a pharmacophore having the following field points:

Atom Type	X	Y	Z	Charge
-7	-1.381791	-0.051772	1.715311	-14.7214
-7	4.879077	-2.537416	-5.803063	-6.0644
-7	-4.418141	0.089392	6.975651	-4.3289
-7	3.562676	4.774324	6.500367	-8.6588
-7	7.206078	1.839680	-1.274007	-8.5662
-7	-6.492228	-10.028677	1.675134	-2.1906
-7	-6.899206	-6.415226	2.683271	-7.7529
-7	-4.444944	-7.302401	-3.535873	-4.6301
-7	-7.304530	8.956919	0.177705	-2.2923
-6	-6.831137	-4.965901	5.561902	2.3235
-6	5.201669	0.095870	-2.055756	8.2589
-6	4.797647	5.131311	-2.954147	6.4282
-6	4.724869	0.054068	5.399575	5.1285
-6	-1.074833	-1.256638	-0.461180	8.2910
-6	5.790484	3.896064	1.509006	3.2358
-6	-5.261753	1.922220	4.363908	6.6584
-6	-1.473675	5.651587	0.238804	6.5755
-6	-5.881620	-0.674191	-5.583631	3.9685
-6	0.611350	-0.417927	5.020914	7.3589
-6	-5.783878	3.835954	-4.612012	6.1148
-6	1.734998	-0.534123	10.273318	2.7267
-6	7.598676	-2.714864	-1.632125	4.5656
-6	9.587200	-2.109952	-4.796533	4.9354
-6	8.219660	0.966784	-8.134950	5.0419
-6	4.675870	2.821225	-7.802368	4.9785
-6	-1.133006	-9.683890	0.147326	5.2926
-6	-7.544412	-1.565759	0.572997	3.8282
-6	-9.125527	-5.337989	-1.040235	2.6320
-6	-7.119754	-8.783302	-1.247938	3.5238
-6	2.332763	-5.381765	3.370511	12.2559

-6	-6.407646	-2.912221	-3.392133	3.6672
-6	-1.476288	-4.227778	1.803737	7.4663
-6	-9.308277	4.502877	0.694358	2.8187
-6	-2.701459	-6.564950	8.367678	8.4013
-6	-0.104383	5.439674	7.873184	2.2847
-5	0.780038	0.691121	1.882956	-11.5525
-5	-6.232273	2.601393	1.696347	-10.7761
-5	-3.467500	-2.270888	3.913391	-11.2323
-5	5.578010	-4.081225	0.238672	-3.5105
-5	-0.161253	0.037560	-6.019390	-8.1238
-5	-2.238659	-0.779217	8.042641	-8.1531
-5	-0.744474	-6.033126	-0.571065	-8.8733
-5	-1.279436	-5.982479	3.824223	-10.0235
-5	-7.480469	-2.520825	0.141700	-8.1691
-5	-1.934318	6.335472	6.394043	-5.5024
-5	-3.708762	5.991115	-0.484472	-6.8736
-5	3.115965	-2.167775	-0.311654	-3.9490
-5	7.338803	2.696229	-2.884882	-5.4513
-5	2.050096	4.896177	5.076291	-7.9992
-5	-4.193728	-2.180720	-3.354927	-7.2592
-5	-4.348258	1.087337	-5.092674	-8.1012
-5	-0.521176	2.639100	10.414149	-3.5695

wherein atom type describes the nature of the field point, -7 represents surface field points, -6 represents positive field points and -5 represents negative field points; X, Y and Z represent co-ordinate values; and charge represents the effective size of the field.

The pharmacophores of this invention can be shown to possess a good relation with MDR modulatory activity. This can be demonstrated by comparing the pharmacophore with the field points of each of the plurality of compounds from which the pharmacophore is derived (already available as a cosmic.fst file) using the overlaying comparison method used previously. The comparison of the field points of each compound with the pharmacophore gives a coulombic overlay energy value. This value can then be correlated with the bioactivity of the compound. The most preferred way of measuring how well the pharmacophore correlates to activity is to plot log [accumulation IC_{50}] against coulombic overlay

calculating the sample correlation coefficient (r). Preferred pharmacophores of this invention, when exposed to this procedure, have an (r) value of not less than 0.5 preferably not less than 0.65.

Pharmacophores of this invention can be used as a tool for screening a molecule for MDR modulatory activity. One such method of screening a molecule for MDR modulatory activity comprises;

(i) obtaining field points describing the positive, negative and van de Waals surface around the molecule;

(ii) measuring the energy of overlay when the molecular field points obtained in (i) are fitted into a pharmacophore of the invention; and

(iii) selecting the molecule as a candidate MDR modulator if the energy of overlay mentioned in (ii) is equal to or lower than the energy of overlay between field points for molecules known to possess MDR modulatory activity and the said pharmacophore.

Pharmacophores of this invention can also be used as a tool for designing a molecule having MDR modulatory activity. One method of designing a molecule having MDR modulatory activity comprises the step of determining the structure of a compound which has field points that have, when fitted into a pharmacophore of the invention, an energy of overlay equal to or lower than a pre-determined value. The pre-determined value can be arbitrarily set or can be the energy of overlay measured when the field points for a plurality of molecules known to possess MDR modulatory activity are fitted into the said pharmacophore.

Compounds which possess MDR modulatory activity can be identified using a pharmacophore of the invention. MDR modulatory compounds can be identified by the method mentioned herein or by any other method using pharmacophores of this invention. Compounds which possess MDR modulatory activity can be designed either by using a method as mentioned herein or by any other method using pharmacophores of the invention.

Examples

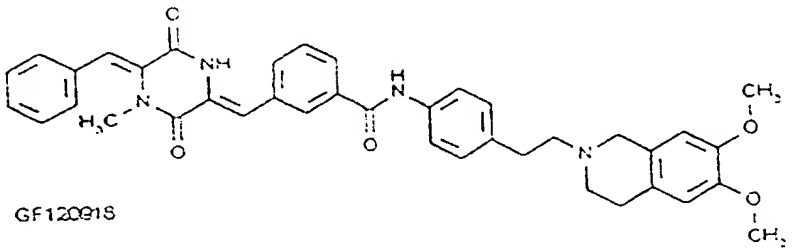
The invention is illustrated by way of the following examples with reference to Figures 1 to 6.

The pharmacophores were produced using "cosmic" molecular modelling suite comprising "xedminlin", "xedfldprep", "xedmin01", "xedqmfcom", "proc-bmf", "xedsort" and "xeddraw", running on a Silicon Graphics Indy, R4400, 150MHZ, 64MB RAM, 2GB HD equipped with XZ Graphics and running IRIX 5.3 operating system.

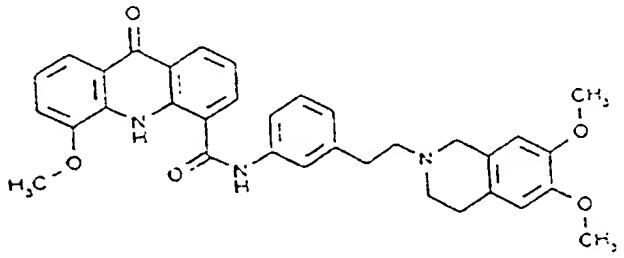
Example 1

The following potent MDR modulators which exhibit a wide range of structural types were used to produce a pharmacophore of the invention.

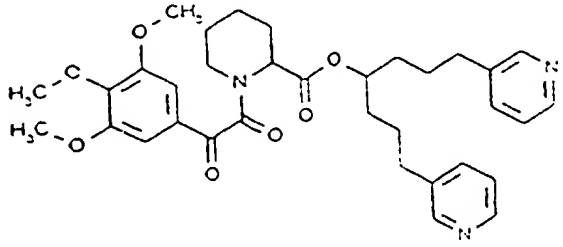
XR9051



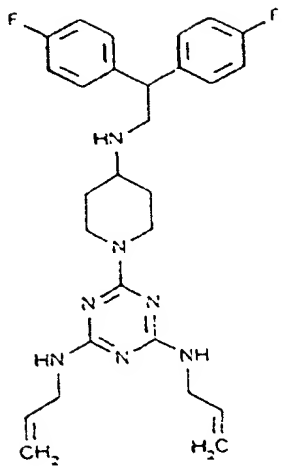
GF120918



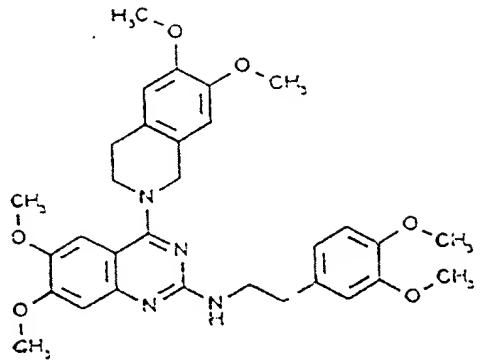
VX-710



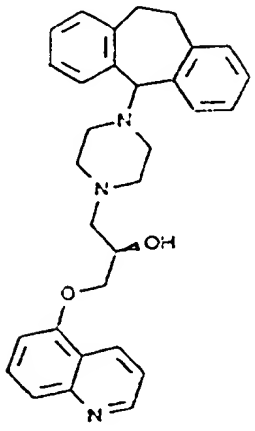
S9788



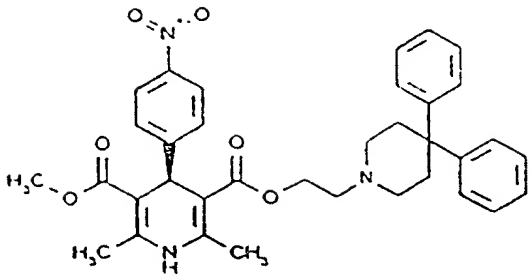
CP100356



CP117227 / MS-073



B8509-035



The structure of each molecule was independently drawn using "xeddraw". Each molecule was then subjected to conformational hunting using "xedminlin". The starting structures were imported into "xedminlin" as cosmic.dat files. The parameters were set as follows:-

Bond increment angle	10°
Upper energy limit above global min	20 Kcal
Number of randomisations	200
Dielectric constant	1
Number of randomisations of flexible ring	5
Hold carbon carbon double bond as entered	Yes
Hold amide carbon nitrogen bond as entered	Yes
Probe for field generation:	peptide/peptide interface.

The program "xedminlin" automatically conformation hunted the molecules, added XED charges and calculated the field points for the 40 lowest energy conformations. A number of lowest energy conformations were then selected for comparison. Table 1 shows the number of conformations selected for each molecule and the energy range above global minimum in which they fall.

Table 1

Compound	Number of Conformers	Energy Range above Global Minimum (Kcal/mol)
XR9051	11	3.17
MS-073	21	3.49
GF120918	7	2.68
CP100356	11	5.26
S9788	21	1.26
VX-710	22	2.99
B8509-035	20	2.93

"xedminlin" automatically links the programs "xedmin01" and "xedfldprep". Conformations including those with XED charges and field points added can be visualized in "xeddraw".

Figure 1 depicts a conformation of XR9051 with XED charges added.

Field points describing the positive, negative and

van der Waals surface around the molecule were defined for each conformation using the program "xedfldprep". Figure 2 depicts a conformation of XR9051 with related field points. In Figure 2, red represents a positive field point, green represents a negative field point and yellow represents a van der Waals surface field point.

Comparisons between pairs of molecules (21 in total) were made, solely on the basis of field point descriptors, for each conformation in the sets using the program "xedqmfcom". "BCOM" was selected from the menu of "xedfldprep". This initiate "xedqmfcom". The names of the two files (cosmic.fst files) to be compared were entered. The results of the comparisons were expressed as coulombic overlay energy between field patterns and ranked in energy order lowest first using the program "proc_bmf". The 50 lowest energy overlays were kept. Table 2 shows the top six ranked overlays and related conformational numbers from the comparison of XR9051 and GF120918.

Table 2

Coulombic Overlay Energy	XR9051 Conformation Number	GF120918 Conformation Number	Order by Coulombic Overlay
-3945.5	8	6	1
-3823.7	8	7	2
-3685.2	8	5	3
-3639.1	2	6	4
-3577.6	8	3	5
-3388.8	8	1	6

The results of the comparisons were mapped onto a conformational mapping diagram and the degree of correlation between the field patterns of specific conformations analysed using the program "xedsort". Figure 3 shows a conformational mapping diagram describing the top 3 links between molecules. This reveals a high correlation between the field patterns of specific conformations. No more than four conformations from each molecule were sufficient to define the best three comparisons between each pair of the seven compounds used.

The optimum cyclic path between molecules was then defined also using the program "xedsort". This facilitated

discrimination between conformers of each molecule on the optimum cyclic path. Figure 4 depicts the optimum cyclic path and related conformations. The conformations that appear on the cyclic path were termed "active" conformations.

The "active" conformations were calculated to be

XR9051	Conformation No.	8
GF120918	Conformation No.	6
MS-073	Conformation No.	10
S9788	Conformation No.	20
B8509-035	Conformation No.	5
VX-710	Conformation No.	22
CP100356	Conformation No.	2

Using the field points of the "active" conformations, available from the corresponding cosmic.fst files, a pharmacophore was obtained using the "create template" function within the program "xedsort". The parameters used in creating the aggregate average were:

Electronic hunting distance	3.0
Surface hunting distance	3.0
Lower limit filter	2.0

The pharmacophore obtained has the following parameters:

Atom Type	X	Y	Z	Charge
-7	-1.381791	-0.051772	1.715311	-14.7214
-7	4.879077	-2.537416	-5.803063	-6.0644
-7	-4.418141	0.089392	6.975651	-4.3289
-7	3.562676	4.774324	6.500367	-8.6588
-7	7.206078	1.839680	-1.274007	-8.5662
-7	-6.492228	-10.028677	1.675134	-2.1906
-7	-6.899206	-6.415226	2.683271	-7.7529
-7	-4.444944	-7.302401	-3.535873	-4.6301
-7	-7.304530	8.956919	0.177705	-2.2923
-6	-6.831137	-4.965901	5.561902	2.3235
-6	5.201669	0.095870	-2.055756	8.2589
-6	4.797647	5.131311	-2.954147	6.4282
-6	4.724869	0.054068	5.399575	5.1285
-6	-1.074833	-1.256638	-0.461180	8.2910
-6	5.790484	3.896064	1.509006	3.2358

	-6	-5.261753	1.922220	4.363908	6.6584
	-6	-1.473675	5.651587	0.238804	6.5755
	-6	-5.881620	-0.674191	-5.583631	3.9685
	-6	0.611350	-0.417927	5.020914	7.3589
	-6	-5.783878	3.835954	-4.612012	6.1148
	-6	1.734998	-0.534123	10.273318	2.7267
	-6	7.598676	-2.714864	-1.632125	4.5656
	-6	9.587200	-2.109952	-4.796533	4.9354
	-6	8.219660	0.966784	-8.134950	5.0419
	-6	4.675870	2.821225	-7.802368	4.9785
	-6	-1.133006	-9.683890	0.147326	5.2926
	-6	-7.544412	-1.565759	0.572997	3.8282
	-6	-9.125527	-5.337989	-1.040235	2.6320
	-6	-7.119754	-8.783302	-1.247938	3.5238
	-6	2.332763	-5.381765	3.370511	12.2559
	-6	-6.407646	-2.912221	-3.392133	3.6672
	-6	-1.476288	-4.227778	1.803737	7.4663
	-6	-9.308277	4.502877	0.694358	2.8187
	-6	-2.701459	-6.564950	8.367678	8.4013
	-6	-0.104383	5.439674	7.873184	2.2847
	-5	0.780038	0.691121	1.882956	-11.5525
	-5	-6.232273	2.601393	1.696347	-10.7761
	-5	-3.467500	-2.270888	3.913391	-11.2323
	-5	5.578010	-4.081225	0.238672	-3.5105
	-5	-0.161253	0.037560	-6.019390	-8.1238
	-5	-2.238659	-0.779217	8.042641	-8.1531
	-5	-0.744474	-6.033126	-0.571065	-8.8733
	-5	-1.279436	-5.982479	3.824223	-10.0235
	-5	-7.480469	-2.520825	0.141700	-8.1691
	-5	-1.934318	6.335472	6.394043	-5.5024
	-5	-3.708762	5.991115	-0.484472	-6.8736
	-5	3.115965	-2.167775	-0.311654	-3.9490
	-5	7.338803	2.696229	-2.884882	-5.4513
	-5	2.050096	4.896177	5.076291	-7.9992
	-5	-4.193728	-2.180720	-3.354927	-7.2592
	-5	-4.348258	1.087337	-5.092674	-8.1012
	-5	-0.521176	2.639100	10.414149	-3.5695

wherein; atom type describes the nature of the field point, -7 represents surface field points, -6 represents positive

field points and -5 represents negative field points; X, Y and Z represent co-ordinate values; and charge represents the effective size of the field. Figure 5 is a pictorial representation of the pharmacophore.

In order to show that the pharmacophore produced is useful for predicting the MDR modulatory activity of a molecule, the field points derived from each of the seven known potent MDR modulators used at the outset were compared with the pharmacophore. The comparisons were again made solely between field point descriptors using the program "xedqmfcom" and the results processed using the program "proc_bmf".

The activities of the MDR modulatory compounds, both accumulation IC_{50} and potentiation IC_{50}, were then obtained either from the literature or by the following assays.

Assays For Testing Activity of MDR Modulatory Compounds

Materials and Methods

The EMT6 mouse mammary carcinoma cell line and the MDR resistant subline AR 1.0 were cultured in RPMI 1640 medium containing 10% foetal calf serum and 2mM glutamine at 37°C in 5% CO_2. Cells were passaged between 1 in 200 and 1 in 2000 in the case of the parental cell line and between 1 in 20 and 1 in 200 in the case of the MDR resistant subline, after trypsinisation (0.25% trypsin, 0.2gl^{-1}, EDTA).

1. Drug accumulation assay

AR 1.0 cells were seeded into 96 well opaque culture plates (Canberra Packard). The assay medium contained a mixture of tritiated Daunorubicin (DNR), a cytotoxic agent, and unlabelled DNR (0.3 μ Ci/ml; 2μM). The MDR modulators were serially diluted in assay medium over a range of concentrations from 5 nM to 100 μM. The cells were incubated at 37°C for 1 hr before washing and determination of cell associated radioactivity. Results are expressed as % maximum accumulation where 100% accumulation is that observed in the presence of the known RMA verapamil at a concentration of 100 μM or as an IC_{50}.

2. Potentiation of Doxorubicin Toxicity

The MDR modulators were examined for their ability

to potentiate the toxicity of doxorubicin in AR 1.0 cells. In initial proliferation assays compounds were titrated against a fixed concentration of doxorubicin (0.86 μM) which alone is non-toxic to AR 1.0 cells. After a four day incubation with doxorubicin proliferation was measured using the colorimetric sulphorhodamine B assay (Skehan et al; J. Natl. Cancer Inst. 82 pp 1107-1112 (1990)).

Compounds which were shown to be able to sensitise AR 1.0 cells to 0.86 μM doxorubicin without high innate toxicity were selected for further study. Cells were cultured for four days with concentrations of doxorubicin over the range of 0.01 nM to 50 μM in the presence of fixed concentrations of MDR Modulator at their maximum subtoxic levels determined from previous experiments. Proliferation was quantified as described by Skehan et al, loc cit. The IC_{50} (concentration required to reduce proliferation to 50% of the untreated controls) for doxorubicin alone and for the MDR Modulators were derived and used to calculate the potentiation index (PI):

$$PI = \frac{IC_{50} \text{ for Doxorubicin alone}}{IC_{50} \text{ for Doxorubicin plus modulator}}$$

Table 3

Compound	Accumulation IC_{50} nM	Potentiation IC_{50} nM	Data Source
GF120918	5-33	8-20	1,2
S9788	32	1580	1
CP100356	160	300	2,3
MS-073	1000	~200	4,3
B8509-035	200	1000	1
VX-710	2500	~2000	2
XR9051	180	200	1

Data Source

1 Boer R and Geketer V, Drugs Fut. 1995, 20, 499-509

2 Assays described herein

3 Kajiji S. et al Biochemistry 1994, 33, 5041-5048

4 Sato W. et al Cancer Res. 1991, 51 2420-2424

Log [Accumulation IC_{50}] was then plotted against coulombic overlay energy for each of the modulators and the sample correlation coefficient(r) calculated by standard means see "statistics" by H.T. Hayslett, Heinemann Professional Publishing, chapter IX.

This gave a correlation of r=0.77.

Figure 6 shows the plot of log [accumulation IC_{50}] against coulombic overlay energy demonstrating good correlation between activity and "fit" in the pharmacophore. The (r) value for log [potentiation IC_{50}] plotted against coulombic overlay energy was 0.87.

Examples 2 to 11

The "active" conformations were obtained as in Example 1. Using the field points of the active conformations available from the corresponding cosmic.fst files, pharmacophores were obtained using the "create template" function within the program "xedsort". Table 4 lists for each example the parameters used in generating the pharmacophore and the sample correlation coefficients when Log [accumulation IC_{50}] and Log [potentiation IC_{50}] are plotted against coulombic overlay energy.

Table 4

Example No	EHD	SHD	LLF	(r) Log [acc IC_{50}] vs coulombic overlay energy	(r) Log [pot IC_{50}] vs Coulombic overlay energy
2	2.0	0.5	2.0	0.54	0.63
3	2.0	3.0	2.0	0.79	0.85
4	3.0	0.5	2.0	0.54	0.63
5	3.0	1.0	2.0	0.54	0.63
6	2.0	0.5	5.0	0.56	0.65
7	2.0	1.0	5.0	0.55	0.69
8	2.0	2.0	5.0	0.72	0.71
9	3.0	0.5	5.0	0.56	0.65
10	3.0	1.0	5.0	0.59	0.69
11	3.0	3.0	5.0	0.77	0.80

C L A I M S

1. A pharmacophore comprising a 3-dimensional array of field points defining a shape and volume wherein the set of 3-dimensional field points is the aggregate average of the field points derived from a plurality of molecules which possess multi drug resistance (MDR) modulatory activity.

2. A pharmacophore according to claim 1, wherein the field points are derived by:

(i) subjecting each molecule to conformational hunting to generate a plurality of conformers for each molecule;

(ii) applying extended electron density (XED) charges to each molecular conformer generated in (i);

(iii) defining field points describing the positive, negative and van der Waals surface around each conformer;

(iv) making an intermolecular energy comparison between the field points defined in (iii);

(v) obtaining the three lowest energy comparisons for each intermolecular comparison and mapping them on a conformational mapping diagram;

(vi) defining an optimum cyclic path which relates the molecules; and

(vii) defining the field points for the conformer of each molecule which lies on the optimum cyclic path defined in (vi).

3. A pharmacophore according to claim 1 or 2 wherein the plurality of molecules which possess MDR modulatory activity includes one or more of XR9051, MS-073, GF120918, CP100356, S9788, VX-710 and B8509-035.

4. A pharmacophore according to any one of claims 1 to 3, which when compared by an overlaying method with the field points of each of the plurality of compounds from which the pharmacophore is derived gives coulombic overlay energy values which when plotted against log [accumulation IC_{50}] has a sample correlation coefficient (r) of not less than 0.5.

5. A pharmacophore according to any one of claims 1 to 4 having the parameters

Atom Type	X	Y	Z	Charge
-7	-1.381791	-0.051772	1.715311	-14.7214
-7	4.879077	-2.537416	-5.803063	-6.0644
-7	-4.418141	0.089392	6.975651	-4.3289

-7	3.562676	4.774324	6.500367	-8.6588
-7	7.206078	1.839680	-1.274007	-8.5662
-7	-6.492228	-10.028677	1.675134	-2.1906
-7	-6.899206	-6.415226	2.683271	-7.7529
-7	-4.444944	-7.302401	-3.535873	-4.6301
-7	-7.304530	8.956919	0.177705	-2.2923
-6	-6.831137	-4.965901	5.561902	2.3235
-6	5.201669	0.095870	-2.055756	8.2589
-6	4.797647	5.131311	-2.954147	6.4282
-6	4.724869	0.054068	5.399575	5.1285
-6	-1.074833	-1.256638	-0.461180	8.2910
-6	5.790484	3.896064	1.509006	3.2358
-6	-5.261753	1.922220	4.363908	6.6584
-6	-1.473675	5.651587	0.238804	6.5755
-6	-5.881620	-0.674191	-5.583631	3.9685
-6	0.611350	-0.417927	5.020914	7.3589
-6	-5.783878	3.835954	-4.612012	6.1148
-6	1.734998	-0.534123	10.273318	2.7267
-6	7.598676	-2.714864	-1.632125	4.5656
-6	9.587200	-2.109952	-4.796533	4.9354
-6	8.219660	0.966784	-8.134950	5.0419
-6	4.675870	2.821225	-7.802368	4.9785
-6	-1.133006	-9.683890	0.147326	5.2926
-6	-7.544412	-1.565759	0.572997	3.8282
-6	-9.125527	-5.337989	-1.040235	2.6320
-6	-7.119754	-8.783302	-1.247938	3.5238
-6	2.332763	-5.381765	3.370511	12.2559
-6	-6.407646	-2.912221	-3.392133	3.6672
-6	-1.476286	-4.227778	1.803737	7.4663
-6	-9.308277	4.502877	0.694358	2.8187
-6	-2.701459	-6.564950	8.367678	8.4013
-6	-0.104383	5.439674	7.873184	2.2847
-5	0.780038	0.691121	1.882956	-11.5525
-5	-6.232273	2.601393	1.696347	-10.7761
-5	-3.467500	-2.270888	3.913391	-11.2323
-5	5.578010	-4.081225	0.238672	-3.5105
-5	-0.161253	0.037560	-6.019390	-8.1238
-5	-2.238659	-0.779217	8.042641	-8.1531
-5	-0.744474	-6.033126	-0.571065	-8.8733
-5	-1.279436	-5.982479	3.824223	-10.0235
-5	-7.480469	-2.520825	0.141700	-8.1691
-5	-1.934318	6.335472	6.394043	-5.5024

-5	-3.708762	5.991115	-0.484472	-6.8736
-5	3.115965	-2.167775	-0.311654	-3.9490
-5	7.338803	2.696229	-2.884882	-5.4513
-5	2.050096	4.896177	5.076291	-7.9992
-5	-4.193728	-2.180720	-3.354927	-7.2592
-5	-4.348258	1.087337	-5.092674	-8.1012
-5	-0.521176	2.639100	10.414149	-3.5695

wherein; atom type describes the nature of the field point, -7 represents surface field points, -6 represents positive field points and -5 represents negative field points; X, Y and Z represent co-ordinate values; and charge represents the effective size of the field.

6. A method of screening a molecule for MDR modulatory activity, which method comprises;

(i) obtaining field points describing the positive, negative and van der Waals surface around the molecule;

(ii) measuring the energy of overlay when the molecular field points obtained in (i) are fitted into a pharmacophore as defined in any one of claims 1 to 5; and

(iii) selecting the molecule as a candidate MDR modulator if the energy of overlay measured in (ii) is equal to or lower than the energy of overlay between field points for molecules known to possess MDR modulatory activity and the said pharmacophore.

7. A method of designing a molecule having MDR modulatory activity, which method comprises the step of determining the structure of a compound which has field points that have, when fitted into a pharmacophore as defined in any one of claims 1 to 5, an energy of overlay equal to or lower than a predetermined value.

8. A method according to claim 7 wherein the predetermined value is the energy of overlay measured when the field points for a plurality of molecules known to possess MDR modulatory activity are fitted into the said pharmacophore.

9. A compound which possesses MDR modulatory activity identified using a pharmacophore as defined in any one of claims 1 to 5 or by a method as defined in claim 6 or designed using a method as defined in claim 7 or 8.

The Patent Office

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Application No: GB 9618177.1
Claims searched: 1-9

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Date of search: 4 November 1997

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Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.O): G4A (AUB, AUXX)

Int Cl (Ed.6): G06F 17/50

Other: Online: WPI, INSPEC, SCISEARCH, EMBASE

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	GB 2266391 A (CRAMER et al.)	1
X, &	US 5307287 A (CRAMER et al.)	1
X	SciSearch Genuine Article No. LL267 & Journal of Computer Aided Molecular Design Vol. 7, No. 3, June 1993, T Langer et al., "Inhibitors of prolyl endopeptidase - characterization of the pharmacophoric pattern using conformational-analysis and 3d-QSAR", pages 253-262 (see abstract)	1

X Document indicating lack of novelty or inventive step
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E Patent document published on or after, but with priority date earlier than, the filing date of this application.